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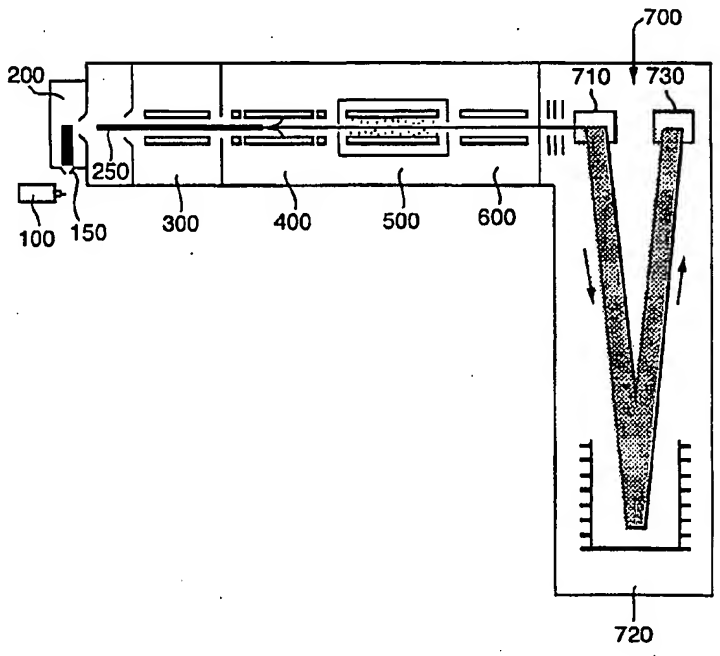
(57) Abstract

An electrospray ionisation probe (100) which comprises a piezoelectric micropump for receiving two liquid components, mixing them to form a sample and ejecting them into a capillary needle which, in turn, emits the mixture as an ionised spray to a mass spectrometer for analysis. The drive voltage for the piezoelectric micropump can be varied to vary the mixing parameters. Temperature regulation of the sample is provided by a thermoelectric device in the probe. Bath gas can be passed through channels of an aluminum block attached to the thermoelectric device to provide temperature controlled gas which is supplied to an apron surrounding the sampling cone of the mass spectrometer. This provides temperature regulation of the sample. Another aspect of the invention provides a method of collecting macromolecular assemblies by using a time-of-flight mass spectrometer in which the ion beam containing packet, containing the macromolecular assemblies is diverted by an orthogonal accelerator (910). The diverted ions are collected on a collector plate (920) which can be an EM grid or X-ray plate, to be used for further analysis. A double isolation valve arrangement is used so that the collection plate (920) can be removed without venting the interior of the mass spectrometer. Macromolecules can be detected and collected intact by admitting gas into the mass analyser to effect collisional cooling namely reducing the internal energy of the ionized macromolecular assemblies.



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IMPROVEMENTS IN OR RELATING TO MICROFLUIDIC SAMPLE PREPARATION AND MASS SPECTROMETRY

The present invention relates to the preparation of samples for solution-based chemical analysis and also to improvements in mass spectrometry.

There is now broad range of techniques and instrumentation used in the analytical sciences for determining all manner of physical and chemical parameters of samples. Some of the main disadvantages of current chemical analysis of biological systems, especially for liquid phase samples, include too slow a transport of the sample in the analysis system, and poor speed (in the case of liquid chromatography or electrophoresis or conventional continuous flow electrospray mass spectrometry). It is desirable, therefore, to improve these techniques by speeding them up, and in particular enabling them to record spectra of interacting solutions, i.e. conformational changes and enzyme reaction mechanisms.

One of the techniques mentioned above, namely mass spectrometry, has been used for analysis of large multi-protein complexes, or macromolecules. The term "macromolecule" or "macromolecular complex" means two or more biological molecules held together by non-covalent interactions. The overall size of such complexes is typically between a few kDa and several mega Da. They include antigens, antibodies, viruses and antigen-antibody interactions. The analysis of such macromolecules using mass spectrometry is, however, difficult. Large proteins analysed from aqueous solutions at neutral pH are often not sufficiently charged to lie within the mass-to-charge range of most commercial mass spectrometers (typically up to 3000 or 4000 m/z). This limitation has been overcome with the introduction of time-of-flight mass analysers coupled with electrospray ionisation. Electrospray can be combined with an orthogonal time-of-flight mass analyser with gated ion flow in which accelerator electrodes are pulsed to extract ions orthogonally from the continuous ion beam. Using these techniques the analysis of molecules with mass-to-charge ratios up to 10,000 have been achieved. However, the larger the protein complex becomes, the more difficult it is to focus and detect the ion beam.

Furthermore, mass spectrometry is essentially a destructive analysis

technique. Thus it is not possible to use mass spectrometry to detect and also collect ions which have been separated according to their mass-to-charge ratio.

According to a first aspect the present invention provides an electrospray ionisation probe which overcomes the problem of slow speed mentioned above. In particular, it provides a sample input probe for an analysis device, which comprises a microfluidic mixer for receiving at least two liquid components and mixing them together to form a sample and means to output the sample to the analysis device.

The sample can be output via a capillary needle and emitted as an ionised spray.

The use of the microfluidic mixer means that the volume of reagents used is very small, and also the delay between mixing and emission from the mixer is very short, for instance 50 milliseconds. The microfluidic mixer can be a piezoelectric micromixer which can have at least two inlet microchannels for receiving the respective components and a mixing chamber. In one such device the mixing chamber is fed by one of the inlet microchannels and outputs through an output channel which is joined by the other of the inlet channels. When the piezoelectric device is driven, the mixing chamber draws the second component into the mixing chamber to mix with the first component which is already there. Then the mixture is ejected to the capillary needle.

The electrical drive for the piezoelectric device can be varied in amplitude, frequency and pulse width to achieve the desired mixing parameters. For instance, the mixing ratio can be varied, and also the timing of mixing and ejection. For example, when the device of one embodiment is driven by a square wave with the frequency of one kilohertz and an amplitude of about 80 volts, it produces a 50:50 mixing ratio and ejects sample within 50 milliseconds of it being mixed.

In one embodiment the volume of the channels and mixing chamber in the micromixer is in the region of 1.1 microlitres.

The speed with which the sample is emitted makes the probe very useful for real-time analysis of reactions. When the mixture has been emitted from the capillary needle as an ionised spray into the gas phase, the reaction between the two components effectively ceases. Thus the very short time between mixing and

emission allows reactions to be closely monitored. For instance, the probe is particularly suitable for supplying sample to a (quadrupole) time-of-flight spectrometer which can then be used to analyse closely the progress of the reaction between the two components.

Advantageously the probe of the invention includes a thermal controller to allow the temperature of the probe to be set as desired, for instance from -4° to +50°C. Further, the probe preferably comprises a gas tight housing for the microfluidic mixer, the housing being penetrated by the capillary needle. The use of the microfluidic mixer allows the output of the mixer to be aligned very closely with the input end of the capillary needle such that, in one embodiment, the distance from the mixer to the distal end of the capillary needle is no more than 15 mm.

Preferably sample is supplied to the mixer by using a rheodyne injector, which eliminates any problems which could be caused by air bubbles.

For electrospray the capillary needle must be charged to a high voltage (e.g. 1000 volts) so the needle should be electrically isolated from the microfluidic mixer and have a conductive coating, e.g. of gold, or have a conductive sleeve thereon.

The use of this probe allows the investigation of the time process of, for instance, enzyme reactions which proceed very quickly. It allow the reactants to be mixed and analysed soon afterwards. Conventional instruments have a dead time from mixture to analysis of one to two minutes. However, the present invention allows the dead time to be reduced to milliseconds. This is achieved by use of the piezoelectric micromixer of small dimensions, placed close to the capillary needle which produces the ionised spray. The distance between the micromixer outlet and the tip of the capillary is short (less than 15 mm), further improving the speed with which the reactants can be brought into the gas phase after mixing.

The invention also provides for the control of the temperature of samples supplied from an electrospray ionisation probe to the analysis device, e.g. mass spectrometer. This is achieved by providing an electrospray ionisation probe with a thermoelectric temperature controlling device and a temperature sensor for sensing the temperature of the probe. Feedback control from the sensor can be used to control the energisation of the thermoelectric device to set the temperature of the

probe as desired. The thermoelectric device is preferably mounted on a thermal block which optionally can have a gas channel within it through which a bath gas for the analysis device can be passed. In the case of a mass spectrometer this bath gas can, for example, be nitrogen or argon. The passing of the bath gas through the thermal block means that the temperature of the bath gas is also controlled by means of the thermoelectric device. The bath gas exiting the block is supplied to the region surrounding the inlet to the analysis device. In the case of a mass spectrometer the sampling cone of the mass spectrometer can be surrounded by an apron which is open towards the inlet of the mass spectrometer and receives the temperature-controlled bath gas. Thus the bath gas flows out from the apron towards the inlet of the sampling cone, in a generally opposite direction to the sample flowing into the sampling cone. The presence of this bath gas regulates the temperature of the region between the outlet of the electrospray ionisation probe and the inlet of the sampling cone. This arrangement can advantageously be used with the electrospray ionisation probe described above.

A second aspect of the invention relates to the use of mass spectrometry not only to measure the mass of macromolecules, or other samples, but also to allow the collection of samples. Thus the invention provides a method of collecting macromolecular assemblies in which the assemblies are ionised and passed through a mass analyser, such as a quadrupole mass filter, to separate them for collection on a collector and optionally simultaneous mass measurement in a second mass analyser, such as a time of flight mass analyser.

The ion beam can be selectively directed to the sample stage or the second mass analyser to provide for alternate collection and detection. The first mass analyser can be quadrupole, time-of-flight, iontrap or magnetic sector.

Advantageously the first mass analyser is one which imparts a relatively low energy to the ions so that they remain intact for collection, while the second can impart a higher energy, which may cause the ions to dissociate but allows detection of the ions or fragments thereof for characterisation. For example, using conventional quadrupoles that usually run at approximately 900 kHz frequency RF supply. Changing the ratio of U/V by dropping the frequency to 300 kHz and

maintaining the same output voltage an anticipated mass range of 32 000 m/z can be achieved.

A maximum resolution of 500 under ideal conditions could be initially achieved, which could easily be used to select specific charge states on, for example, the MS2 virus for selection and detection. In this case, the device can be used for both sample enrichment and mass selection inside the mass spectrometer.

The collector can be an EM or X-ray plate of conventional type which collects the ions having been selected by the first mass analyser. The ion beam can be diverted to the collector by using an orthogonal accelerator. The first mass analyser will allow the transmission of a specified m/z range for collection and alternate detection and mass measurement in the second analyser. By driving the orthogonal accelerator with a square wave, it is possible to alternately divert ions and let them through to the second mass spectrometer thus providing both the detection and collection of sample.

In one embodiment the collector can be disposed in a housing provided in the wall of the mass spectrometer and having sealing means for selectively:

(a) sealing housing from the atmosphere and opening it to the interior of the mass spectrometer to allow collection of sample; and

(b) sealing the housing from the mass spectrometer and opening it to the atmosphere to allow removal of the collector. To achieve this the housing can conveniently comprise two sub-chambers separated from each other by a first isolation valve, one of the chambers containing the collector and the second of the chambers being selectively communicable with the interior of the mass spectrometer via a second isolation valve. The interior of the housing can be connected to a vacuum source to allow its evacuation.

This arrangement allows the sample to be collected and removed without needing to vent the interior of the mass spectrometer.

Thus the invention allows the simultaneous mass analysis, separation and collection inside the mass analyser chamber. The collector plate or grid can be removed without venting the instrument, and structural studies on the assemblies collected on the collector can be performed, e.g. by cryoelectron microscopy or X-

ray diffraction.

Because the ions have been mass filtered, e.g. by a quadrupole analyser this enable specific isolation and collection of individual molecular ions. The invention allows, for the first time, the separation and sample collection on the basis of mass-to-charge using a mass spectrometer.

Of course, in order that the invention can be used for the analysis and, optionally, the collection of macromolecules, it is important to ensure that the macromolecules remain intact in their flight through the mass analyser. This can be achieved by a third aspect of the invention which involves careful control of the pressure and pressure differentials throughout the analyser. When analysing large ions the accelerating voltages used in the source region of the instrument need to be greater than with small ions to give the large ions enough energy to overcome the pressure gradients within the mass spectrometer. Large ions have more sites for protonation (20-200 charges are possible for macromolecules). This, combined with the high accelerating voltages (100 to 200 eV) leads to a high kinetic energy for the ions. However, increasing the kinetic energy of the ions reduces their stability and also can cause them to collide with lenses and rf ion guides. Thus there is a difficult balance between giving the ions enough energy to get them into the mass spectrometer and maintaining their integrity.

Large ions also can carry a high internal energy, such as in rotation, which also increases the likelihood of fragmentation.

The pressure differentials between different parts of the instrument can be adjusted so that the molecules do not encounter large rate of change of pressure. The pressure differentials are adjusted by admitting gas into the chambers at a controllable rate, and adjusting the vacuum pumps exhausting the chambers.

The admission of the gas reduces the high energy collisions of the ions as they pass from atmosphere to low pressure. The ions undergo a larger number of low energy collisions, rather than a few high energy ones.

Further, the introduction of gas into the instrument reduces the internal kinetic energy of the macromolecular complexes by collisional cooling.

Although gas collisions have been used before in specific collision cells

provided along the path of the instrument to a high degree of vacuum, this aspect of the present invention reduces the vacuum in the main chambers of the instrument, e.g. in the chambers housing the first and, optionally, the second R.F. hexapoles. The cooling gas is chosen according to the number of molecular degrees of freedom and the degree of cooling required. Thus a diatomic gas such as nitrogen, or heavier gases such as Argon and Xenon can be used. Cooling gases with more degrees of freedom, such as sulphur hexafluoride or polymers mixed with isobutane, can absorb more energy.

In addition to the admission of such gases to the chamber of the instrument, a conventional collision cell can also be used placed directly on the ion beam path. This opens the possibility of selectively dissociating the macromolecules as they traverse the collision cell. This allows the instrument to be used to investigate sub-unit interactions within the macromolecular assembly.

The invention will be further described by way of non-limitative example with reference to the accompanying drawings, in which:-

Figure 1 is a schematic diagram of a quadrupole time-of-flight mass spectrometer;

Figure 2 is a schematic top cross-sectional view of the electrospray ionisation probe according to an embodiment of the invention;

Figure 2A is a schematic top cross-sectional view of a modification of the probe of Figure 2;

Figure 2B is a cross-sectional view of part of the modified probe of Figure 2A;

Figure 3 is a schematic side view of the electrospray ionisation probe of Figure 2;

Figure 4 is a schematic view of part of the electrospray ionisation probe of Figure 2;

Figure 5 is a schematic view of a microfluidic mixer from the electrospray ionisation probe of Figure 2;

Figure 5A is a cross-section along the line I-I of Figure 5;

Figure 6 is a schematic side view of the face plate of the electrospray

ionisation probe of Figure 2;

Figure 7 is a schematic view of a thermal controller of the electrospray ionisation probe of Figure 2;

Figure 8 schematically shows a front view of the power supply for the electrospray ionisation probe of Figure 2;

Figure 9 is a schematic view of a time-of-flight mass spectrometer according to a second embodiment of the invention;

Figure 10 is a schematic view of a sample collection apparatus according to a second embodiment of the invention;

Figure 11 is a further schematic view of the sample collection apparatus of Figure 10;

Figure 12 schematically illustrates an LCT-t.o.f. mass spectrometer according to a third embodiment of the invention;

Figure 13 schematically illustrates a Q-t.o.f. mass spectrometer according to a third embodiment of the invention;

Figure 14 shows mass-spectra obtained by use of a third embodiment compared with conventional results;

Figure 15 shows schematically two mass analysers used with a sample collector;

Figure 16(a) and (b) illustrate mass spectra of the MS2 virus obtained with embodiments of the inventions; and

Figure 17 illustrates spectra of the MTGIMC chaperone at different temperatures obtained using the invention.

First Embodiment

Referring to Figure 1 there is illustrated a typical quadrupole time-of-flight mass spectrometer. It includes an external electrospray ionisation probe 100, whose details will be described later, which supplies the ionised sample to a sampling cone 150 leading into a source region 151 and extraction lens 200. The extraction lens 200 forms an ion beam 250 which is passed through the successive stages of the

mass spectrometer, namely the RF hexapole 300, the quadrupole mass filter 400, the hexapole collision gas cell 500, a further RF hexapole 600 and the time-of-flight tube 700. The time-of-flight tube comprises a pusher 710 for orthogonally pushing the ions to a reflectron 720, where they are reflected to a detector 730.

A first aspect of the invention particularly concerns the external electrospray ionisation probe 100. This is illustrated in detail in Figures 2 to 7. At the heart of the probe 100 is a piezoelectric micromixer 101 formed by a piezo-driven micropump. The micromixer 101 is shown in detail in Figure 5 and comprises two inlets A and B for receiving two liquid components which are to be mixed to form a sample for mass spectrometry. Inlet A leads via an inlet microchannel 1010 to a pump chamber 1014 which therefore forms a mixing chamber. The inlet B leads via a second inlet microchannel 1012 to a junction 1018 with the output channel from the pump chamber 1014. In use the two components mix in the pump chamber 1014 and are output from the outlet 1016. The micropump 101 in this embodiment, which is shown in cross-section in Figure 5A, is a silicon-glass chip whose size is 12.0 x 10.9 x 1.4 mm (width x length x thickness) which thus has a small overall volume. The volume of the pump chamber is 900 nanolitres and the dead volume of the inlet microchannel 1012 is 200 nanolitres. The dead volume of the inlet microchannel 1010 is negligible, thus giving an overall dead volume of only 1.1 microlitres. This small dead volume means that the delay from mixing of the two components to ejection of the mixture from the micromixer is about 50 milliseconds. The microchannels and pump chamber 1014 are formed in a layer 1021 of silicon supported on a glass substrate 1020. Fluids are driven through the device by application of voltages via connections to the piezoelectric membrane which bends the chip.

The mixture is ejected from the device as a droplet stream, the size of single droplets being about 100 micrometres, and travels to the sampling cone 150 of the mass spectrometer (a distance of about 5 mm) and into the source region of the mass spectrometer.

As shown in Figure 2 the micromixer 101 is mounted within an airtight insulated housing 109 on a peek bracket 107. The outlet of the micromixer is aligned

with a nanoflow capillary 103, for instance a borosilicate glass needle of length 20 mm and internal diameter of 1.5 mm at the micromixer tapering down to a fine capillary at the output end. High voltage connection 105 is provided to allow application of the ionisation voltage to the needle. The housing 109 is suppled with nitrogen via inlet 121, and power is supplied for the micromixer via connector 123. In use, the interior of the housing is pressurised to 5 to 30 mba of nitrogen.

The temperature of the interior of the housing and the micromixer can be adjusted as desired. To this end the housing includes a temperature sensor 111 and a thermal controller which consists of a thermoelectric module 115 and a cold block 113. The thermoelectric controller 115 has a heat sink formed by a fan 117. In use the temperature of the device can be set from -4°C to 50°C.

As shown in Figure 3 the probe is mounted on a base 108 via X, Y and Z adjusters 102, 104 and 106. These allow the probe to be aligned as desired with the sampling cone 150 of the mass spectrometer. Sample is suppled to the micromixer 101 by a rheodyne injector 127 mounted on a bracket 119.

As shown in Figure 4, because the probe housing 109 needs to be airtight, the power supply lead for the micromixer, the two inlet tubes for the mixer and the gas inlet for back pressure must maintain a gas tight seal. The gas inlet for back pressure (nitrogen) is interfaced with stainless steel fittings. The power supply lead and two inlet tubes have tight-fitting rubber O rings 122 and 124 around their circumference. The O rings are clamped by a perspex flange 125 which screws into the housing 109 creating an airtight seal around the tubes and lead. Figure 4 does not illustrate the similar arrangement which is used for the power lead for the micromixer.

Figure 6 illustrates schematically how the micromixer is aligned with the borosilicate glass capillary needle 103. The two liquid components which are to be mixed are supplied by inlet tubes 1030 and 1032 to the inlets A and B. The outlet 1016 of the micromixer is aligned with the axial bore of the glass capillary 103. To ionise the sample emitted from the capillary it is necessary to apply a high voltage, for instance of 1000 volts, to it. As shown in Figure 6 the glass capillary 103 is held in place on the housing 109 by a sleeve 1037 of electrically conductive elastomer which in turn is connected to a brass sleeve 1033. The brass sleeve is connected to a

high voltage source. The brass sleeve is surrounded by a peek sleeve 1035 which insulates the capillary 103 from the probe housing. The exterior of the probe housing is anodised to protect against electric shock, and to provide further electrical isolation of the micromixer 101 from the capillary, the micromixer is mounted on the peek bracket 107. This bracket 107 is adjustable to allow the outlet 1016 of the micromixer to be aligned with the capillary 103.

The micromixer can also be used in conjunction with a levitator, which levitates the droplets before entry into the mass analyser. Furthermore, the micromixer does not need to be interfaced with any capillaries, but also has the advantage of being able to inject droplets directly into the mass spectrometer.

By using this microfluidic device, which can be positioned close to the capillary needle, so that the distance from the outlet of the mixer to the outlet of the needle can be no more than 15 mm, it is possible to minimise the deadtime from mixing to ejection from the needle to be only 50 milliseconds.

In the micromixer different ratios of mixing ratio for the two components can be achieved by varying the dimensions of the inlet channel. On energisation of the piezoelectric mixer, the liquid from the second inlet channel 1012 is drawn into the pump chamber 1014 and mixes with the other component which is already in the pump chamber (having been fed from inlet A).

However, it is also possible to vary the parameters of the mixing process by varying the electrical drive to the piezoelectric pump. For instance, in this embodiment at a driving frequency of 1 kilohertz, the mixing ratio achieved by the pump is 50/50. The pump chamber is designed so that at the resonant frequency it takes the same amount of component from each inlet. A schematic front view of the power supply 10 is shown in Figure 8. The piezoelectric pump is driven by a square wave whose amplitude, frequency and pulse width can be varied. In this embodiment the amplitude can be varied between 50 and 80 volts by control 18, the frequency between 0.2 and 2 kilohertz by control 16 and the pulse width between 20 and 250 microseconds by control 20. The time of activation can be varied between 1 and 10 seconds by control 14 and the run button 12 initiates mixing and timing.

As mentioned above the temperature of the probe can also be set as desired

by means of the thermal controller 115 which is shown schematically in more detail in Figure 7. The temperature is set by using a Peltier module consisting of two dissimilar semiconductors 115A and 115B. These can transport heat from the cold block 113 to a heat sink 115C when energised by a power source 115D. Of course, the cold block 113 can actually be heated or cooled, according to the polarity of the voltage applied to the Peltier module. As shown in Figure 8 the temperature of the probe is displayed on display 24 and the desired temperature can be set using the keys 26.

Although the probe above has been described using a micromixer having two inlet channels for mixing two components, the micromixer can, of course, be adapted to have three inlet channels (or more) for mixing more components.

Further, although the probe has been illustrated as inputting to a quadrupole time-of-flight mass spectrometer, it can be used as an input to any mass spectrometer or other types of analysis device which require an ionised stream of sample.

Figures 2A and 2B illustrate a modification of the probe which allows the samples to be thermally incubated and delivered into the gas phase at constant temperature. The cold block 113 of the probe of Figure 2 is replaced by a thermal regulator block 113A. The thermal regulator block 113A is preferably made of aluminum. The temperature of this block is controlled by the thermoelectric cooling device 115 to any temperature between 0°C and 120°C, and is provided with a gas channel 1131 running through its interior. The gas that runs through the channel is the bath gas, for instance nitrogen or argon, and this flows from an inlet 1132, through the gas channel 1131 and to an outlet 1133. It then flows via iso-versinic tubing 1134 to an inlet 1135 provided in an apron 150A around the sampling cone 150 of the mass spectrometer. This gas, whose temperature is controlled by virtue of having passed through the thermal regulator 113A flows in general in the opposite direction to the sample coming from the capillary 103.

Thus this allows the temperature of the region between the end of the capillary 103 and the entrance to the sampling cone to be thermally regulated, so as to maintain a constant, desired temperature. This allows reagents to be dissociated or maintained thermally under native conditions, which provides valuable kinetic and

structural information.

It will be appreciated that the provision of the thermal regulator 113A and the apron 150A providing the counter-current gas stream can be used with other types of electrospray ionisation probe. Their use is not limited to the probe described above and illustrated in Figure 2.

Figure 17 illustrates spectra of the MTGIMC chaperone at temperatures from 40°C to 70°C illustrating disappearance of the complex as the temperature of the sample is increased.

Second Embodiment

A second aspect of the invention concerns the use of a mass spectrometer for collection of sample, in particular of macromolecules. As mentioned above, mass spectrometry is usually a destructive analysis technique and so it cannot be used to collect sample. Figures 9 to 11 illustrate schematically how this embodiment of the invention adapts the mass spectrometer to allow sample collection.

Figure 9 schematically shows a typical time-of-flight mass spectrometer. Sample is injected from probe 100 into a focussing lens 300 which produces an ion beam 350. The beam 305 is reflected by reflectron 800 to a detection/collection region 900. This includes a conventional detector 905, and an apparatus for collecting samples from the beam, (910 and 920). The apparatus for collecting the sample consists of an orthogonal accelerator 910 which is energised by a rectangular push-out pulse 912 as illustrated in Figure 11. Thus ions from the beam 350 are alternatively allowed to pass to the detector 905, or diverted to the collection grid 920 attached to the collection probe and which is either a standard cryo-probe or probe with translational adjustments available. At this point in the instrument, the ions have been separated according to their mass-to-charge ratio by the first mass analyser such as a quadrupole mass filter. The molecular ions are then alternately pulsed with an orthogonal accelerator and collected onto grids. Where electrospray ionisation is used, the ion beam inside the mass spectrometer is continuous, which allows the ions to be collected and pulsed by the push-out pulse to the collection

plates, alternately with linear detection at the conventional microchannel detector plate 905. As shown in Figure 11, ions are diverted from the ion beam 350 by the accelerator plate and pass through a drift region under the influence of focussing and accelerator lenses 913 and 915 before being collected on collection plate 922. The accelerator plate can be angled slightly (by a few degrees, e.g. 1° or 2°) away from the collector plate along direction of travel of the ions to optimize collection.

Of course, in order to divert the ions it is important that the push-out pulse is synchronised correctly with the gating of the ions in the beam.

The collector plates/grids are positioned on a conventional EM probe which may have a retarding electric potential to reduce the velocity of the ions before they hit the collection grids. At the point where the ions approach the collection grid the dynode detector typically employed as a point detector is removed allowing probe access. The ions collected on the sample gird are either collected onto a number of commercially available grids with a glycerol based adhesive to land ions after selection in the first mass analyser. Alternatively, samples can be collected onto a cryo-cooled probe. The collector plate 922 is formed by a conventional EM grid or X-ray plate (obtainable from routine laboratory suppliers) as desired for subsequent analysis. Of course, it is necessary that the sample collected on the collector plate 922 can be removed easily from the mass spectrometer. This means that plate/grid must be removable without venting the mass spectrometer to atmosphere. With the present invention this is achieved by mounting the collector plate 922 in a housing 921 and mounted in the side 919 of the mass spectrometer which has two sub-chambers 923 and 925 as shown in Figure 10. The collector plate 922 is provided in one of the sub-chambers 923. This sub-chamber is isolatable from the second sub-chamber 925 by an isolation valve 924. The second sub-chamber 925 can be selectively brought into and out of communication with the interior of the mass spectrometer via an isolation valve 926. The sub-chamber 925 can be evacuated by means of a source of vacuum 928.

The use of the two isolation valves 924 and 926, means that the collection plate 922 can easily be removed from the mass spectrometer after sample has been collected without any loss pressure in the interior of the mass spectrometer. When

the collection plate 922 is replaced on the housing, the isolation valve 924 between the two sub-chambers can opened and the interior of the housing evacuated using vacuum source 928. When evacuation is complete, the second isolation valve 926 can be opened to allow further sample to be collected from the mass spectrometer.

It may also be noted that the collector 920 can be manufactured from non-magnetic materials such as aluminum or stainless alloys because it does not rely on magnetism for its functioning. The advantage of this is that it does not affect the magnetic fields in the mass analyser.

The instrument described above is particularly useful for collecting macromolecular assemblies having a high mass/charge ratio (for instance from 100 to 40,000). This technique can be developed further by positioning the collector 920 between two mass analysers, as shown in Figure 15, so that the macromolecular complexes produced at source 1501 are first mass analysed by a first mass analyser 1500 then directed by diverter 918 either to the collector 920 or to a second mass analyser 1502 for measurement at detector 1503. Thus, for instance, the collector 920 can be positioned after a quadrupole mass analyser used to filter the ions, but before a t.o.f. analyser used for detection and mass analysis. Other pairs of analysers which can be used are t.o.f.-t.o.f., iontrap-t.o.f., quadrupole-quadrupole, magnetic sector - t.o.f., or FTICR analysers. In each case the collector is placed in between the analysers to selectively receive macromolecular complexes.

Advantageously the first of the mass analysers can be of a type which imparts a lower energy to the ions than the second. This allows the collection of fragile assemblies which are often fragmented by higher energy analysers. A higher energy may be used for the second analyser, performing detection, if fragmentation does not affect the detection result. An example of a low energy-high energy pair is a quadrupole mass analyser followed by a t.o.f. mass analyser.

Third Embodiment

Figures 12 and 13 illustrate two specific types of mass analyser adapted according to a third aspect of the invention, in particular by providing for the

admission of gases into the analyser. This allows for the pressures and pressure differentials throughout the analyser to be adjusted so that large macromolecular complexes can traverse the instrument without breaking up. Figure 12 illustrates an LCT time-of-flight mass spectrometer in which two gas inlets 30 and 32 are provided for admitting gas into the region of the first hexapole 34 and the second hexapole 36. In the instrument shown in Figure 12 sample is emitted from the external ESI probe 38 and travels via a sample cone 40 and extraction lens 42 into the instrument. Conventionally the regions of the first and second hexapole would be evacuated to a high degree, such as 0.1 mba. However, with the present invention a collisional cooling gas is introduced via the inlets 30 and 32 so that the pressure in these regions is higher than that. This increases the chance of the macromolecular complexes colliding with the gas and losing kinetic, vibrational and rotational energy. The loss of energy tends to increase the lifetime of the macromolecular complexes. Providing for collisional cooling of the ions by admission of gas into the interior of the mass analyser reduces the kinetic and internal energy of the ion and thus increases their stability. The gas pressure used inside the instrument depends on the size of ion being analysed. Pressures used in the LCT-t.o.f. mass spectrometer of Figure 12 (measured using a Penning gauge) are as follows:

1.	base pressure	8.0e-7 mBa
2.	ions up to 100 kDa	1.0e-6 mBa
3.	ions 100-300 kDa	2.0e-6mba
4.	ions 300-800 kDa	8.0e-6 mBa
5.	MDa	4.0e-6 mBa

Figure 13 illustrates a corresponding adaptation for a quadrupole-t.o.f. mass spectrometer. Again, gas inlets 30 and 32 are provided, the first in the region of the first RF hexapole 33 and the second in the chamber occupied by the RF hexapole 33, a quadrupole mass filter 35 and a hexapole collision gas cell 37. It should be appreciated that the hexapole collision gas cell 37 is used primarily to selectively breakup the ions in the beam. The hexapole collision gas cell 37 therefore differs in both function and its operational parameters from the admission of gas via the gas inlets 30 and 32. As illustrated in Figure 13 ions exiting the second RF hexapole 33

travel into a time-of-flight mass analyser which is also provided with a collector 920 to which ions can be selectively diverted by a diverter 910. Typical pressures used in the instrument of Figure 13 are as follows:-

1.	base pressure	analyser 8.6e-6 mBa
	t.o.f.	1.2e-7 mBa
2.	ions up to 300 kDa	analyser 2.0e-5 mba
	t.o.f.	2.2e-7 mBa
3.	ions 300-800 kDa	analyser 8.0e-6 mBa
	t.o.f.	3.7e-7 mBa
4.	MDa	analyser 4.5e-4 mBa
	t.o.f.	7.0e-7 mBa

It should be appreciated that the pressure used will depend on the cross-sectional area of the ion because ions that have large cross-sectional areas will have more collisions. For instance, a virus (with a spherical cross-sectional area) will require a lower pressure than an ion with a small-cross-sectional area such as an antibody. The above pressures were recorded using dry nitrogen as the cooling gas. Other gases, however, can be used. The degree of cooling achieved by a given collision depends on the number of degrees of freedom of the cooling gas. For a monotonic gas there are three degrees of freedom, which are all translational. For a diatomic gas there are five degrees of freedom - three translational and two rotational. Thus more efficient collisions occur with molecules capable of absorbing more energy. Collisional cooling is beneficial even with small diatomic gases such as nitrogen. Heavier gases such as Argon and Xenon can also be used.

The use of heavier gases for cooling allows an increase in a duty cycle of the instrument. Furthermore, using sulphur hexafluoride (where the number of degrees of freedom is 3n-6, giving a total of 15 different types of vibration) was found to give the best sensitivity. Other types of gases are also feasible, including any gas that has a vapour pressure above that of atmosphere. An example is the use of polymers mixed with isobutane, which has a very high number of degrees of freedom.

The use of such collisional gases in the instrument promotes not only the sensitivity of the instrument for larger ions, but is actually essential for the

observation of large macromolecular complexes. Without collisional cooling no ions have been detected for high masses, such as MDa and ions in excess of a few MDa.

With these techniques an aqueous solution (100% water at pH 5.0) of GroEL introduced by nanoflow electrospray can be induced to remain as an intact complex in the gas phase, permitting its observation by mass spectrometry. For instance an assembly of 14 sub-units of GroEL have been induced to remain intact throughout the spectrometer.

Figure 14 illustrate the spectra of a native antibody showing the raw data for identical scans and under identical conditions with different cooling gases admitted via inlets 30 and 32. The value indicated as "t.o.f. MS+" is the total ion current recorded. Higher values mean high duty cycles. It will be seen that the bottom of the three scans, (which uses no cooling) has a very low signal to noise ratio compared to the upper two scans which use sulphur hexafluoride and Xenon as indicated. Thus the effect of the admission of gases into the instrument is to increase the sensitivity to large macromolecular complexes.

Figures 16(a) and (b) show spectra for an intact MS2 virus capsid demonstrating macromolecular transmission through the instrument. Figure 16(a) shows histograms produced from direct ion hits on the detector at (i) 5, (ii) 10, and (iii) 50 ions summation. Figure 16(b) shows smoothed data indicating m/z values of identifiable change states.

CLAIMS

1. An electrospray ionisation probe for supplying sample to an analysis device, the probe comprising a microfluidic mixer for receiving at least two liquid components and mixing them together to form a sample, and a capillary needle for receiving the sample from an outlet of the mixer and emitting it as an ionised spray.

2. An electrospray ionisation probe according to claim 1, wherein the microfluidic mixer comprises a piezoelectric micromixer through which the liquid components are driven by piezoelectric deformation under an applied electrical voltage.

3. An electrospray ionisation probe according to claim 2, wherein the piezoelectric micromixer comprises at least two inlet microchannels into which respective components are drawn upon electrical driving of the micromixer.

4. An electrospray ionisation probe according to claim 3,wherein the piezoelectric micromixer comprises a mixing chamber fed by one of said inlet microchannels, and outputting through an outlet channel which is joined by the other of said inlet microchannels to mix the two components and output the mixture.

5. An electrospray ionisation probe according to claim 2 or any claim dependent therefrom, further comprising an electrical driver for the piezoelectric micromixer, the electrical driver being adapted to vary selectably the drive voltage for the piezoelectric micromixer.

6. An electrospray ionisation probe according to claim 5, wherein the electrical driver is adapted to apply a square wave drive voltage to the piezoelectric micromixer.

7. An electrospray ionisation probe according to claim 5 or 6, wherein the electrical driver is adapted to vary selectably at least one of the frequency, amplitude or pulse width of the drive voltage for the piezoelectric micromixer.

8. An electrospray ionisation probe according to any one of the preceding claims, further comprising a thermal controller for controlling selectably the temperature of the probe.

9. An electrospray ionisation probe according to claim 8 wherein the thermal controller comprises a thermoelectric device attached to a thermal block, and a temperature sensor, the thermoelectric device heating or cooling the thermal block to maintain a predetermined temperature as sensed by the temperature sensor.

10. An electrospray ionisation probe according to claim 9 wherein the thermal block is provided with a gas channel having an inlet for receiving an inert gas and an outlet for supplying the inert gas to a region surrounding the inlet to the analysis device.

11. An electrospray ionisation probe according to claim 10 in combination with a mass spectrometer, wherein the sampling cone of the mass spectrometer is surrounded by an apron receiving said gas and allowing said gas to flow out around the inlet to the sampling cone.

12. An electrospray ionisation probe according to any one of the preceding claims, wherein the microfluidic mixer is provided within a gas tight housing penetrated by the capillary needle, the capillary needle extending from a proximal end aligned with the outlet of the microfluidic mixer, through the gas tight housing to a distal end from which the sample is emitted.

13. An electrospray ionisation probe according to claim 12, further comprising charging means for applying an electrical charge to the capillary

needle, the needle being electrically isolated from the microfluidic mixer.

14. An electrospray ionisation probe according to claim 13, wherein the charging means comprises an electrically conductive sleeve surrounding part of the capillary needle and connected to an electrical power source.

15. An electrospray ionisation probe according to claim 12, 13 or 14, herein the distance from the microfluidic mixer to the proximal end of the capillary needle is no more than 15mm.

16. An electrospray ionisation probe according to any one of the preceding claims, further comprising a Rheodyne injector for supplying the components to the microfluidic mixer.

17. A method of collecting macromolecular assemblies comprising the steps of:

ionising the macromolecular assemblies and passing them through a mass spectrometer to separate them in an ion beam according to their charge-to-mass ratio; and

diverting the ion beam to a collector.

18. A method according to claim 17 comprising the steps of selectively directing ions from the ion beam from the mass spectrometer to a collector or to a second mass spectrometer.

19. A method according to claim 18 wherein the first mentioned mass spectrometer is a quadrupole, time-of-flight, iontrap, magnetic sector or Fourier transform ion cyclotron resonance mass spectrometer and the second mass spectrometer is a time-of-flight mass spectrometer.

20. A method according to claim 18 wherein the first-mentioned and second mass spectrometers are quadrupole mass spectrometers.

21. A method according to claim 17 wherein the ion beam is diverted to the collector by an orthogonal accelerator.

22. A method according to claim 21 wherein the energisation of the orthogonal accelerator is timed in relation to the time-of-flight of the ionised macromolecular assemblies to divert them to the collector.

23. A sample collection apparatus for a mass spectrometer, the apparatus comprising: an orthogonal accelerator for diverting the ion beam of the mass spectrometer to a collector, the collector being disposed in a housing, the housing having sealing means for selectively: a) sealing the housing from the atmosphere and opening it to the interior of the mass spectrometer, and b) sealing the housing from the mass spectrometer and opening it to the atmosphere to allow removal of the collector.

24. A sample collection apparatus according to claim 23, wherein the housing comprises two chambers and a first isolation valve for selectively communicating them with each other, a first of the chambers containing the collector, and the second of the chambers being selectively communicable with the interior of the mass spectrometer via a second isolation valve.

25. A sample collection apparatus according to claim 23 or 24, wherein the interior of the housing is connected to a vacuum source to allow evacuation of the housing.

26. A sample collection apparatus according to claim 23, 24 or 25, wherein the collector is an em or x-ray grid.

27. A sample collection apparatus according to claim 23, 24, 25 or 26 wherein the axis of the orthogonal accelerator is angled by a few degrees away from the collector.

28. A method of mass analysing macromolecules using a mass analyser comprising the step of admitting a collisional cooling gas into the mass analyser during operation thereof to reduce the internal energy of the ionized macromolecular complexes while maintaining them intact.

29. A method according to claim 28 wherein the collisional cooling gas is admitted into the analyser at two locations spaced along the ion beam path.

30. A method according to claim 29 wherein the two locations are in the chambers housing the first and second rf hexapoles respectively.

31. A method according to claim 28, 29 or 30 wherein the collisional cooling gas is selected from nitrogen, argon, xenon, sulphur hexafluoride or a mixture of a polymer with isobutane.

32. A method according to any one of the preceding claims wherein the rate of admission of collisional cooling gas and the rate of exhaustion of gases from the mass analyser are set to give a gas pressure therein in the range 0.5 to 50 mba.

33. A method according to any one of the preceding claims wherein the rate of admission of collisional cooling gas and the rate of exhaustion of gases from the mass analyser are set to give a gas pressure therein in the range 0.5 to 25 mba.

34. A method according to any one of the preceding claims wherein the rate of admission of collisional cooling gas and the rate of exhaustion of gases from the mass analyser are set to give a gas pressure therein in the range 1 to 10 mba.

35. A mass analyser comprising a gas inlet for admitting a collisional cooling gas into the mass analyser to reduce the level of vacuum therein during operation.

36. A mass analyser according to claim 35 wherein there the gas inlet is

provided for admitting collisional cooling gas into the chambers housing the first and/or second rf hexapoles respectively.

37. A mass analyser according to claim 35 or 36 further comprising means for controlling the rate of admission of collisional cooling gas via the gas inlets and the rate of exhaustion of gases from the mass analyser to give a gas pressure therein in the range 0.5 to 50 mba.

38. A mass analyser according to claim 35 or 36 further comprising means for controlling the rate of admission of collisional cooling gas via the gas inlets and the rate of exhaustion of gases from the mass analyser to give a gas pressure therein in the range 0.5 to 25 mba.

39. A mass analyser according to claim 35 or 36 further comprising means for controlling the rate of admission of collisional cooling gas via the gas inlets and the rate of exhaustion of gases from the mass analyser to give a gas pressure therein in the range 1 to 10 mba.

40. A mass analyser according to any one of the preceding claims wherein the collisional cooling gas is selected from nitrogen, argon, xenon, sulphur hexafluoride or a mixture of a polymer with isobutane.

41. An electrospray ionization probe constructed and arranged to operate substantially as hereinbefore described with reference to an as illustrated in Figure 1 to 8 of the accompany drawings.

42. A method of collecting macromolecular assemblies substantially as hereinbefore described with reference to and as illustrated in Figures 9 to 15 of the accompanying drawings.

43. A sample collection apparatus for a mass spectrometer, the apparatus being constructed and arranged to operate substantially as hereinbefore

described with reference to and as illustrated in Figures 9 to 15 of the accompanying drawings.

44. A method of mass analysing macromolecular substantially as hereinbefore described with reference to an as illustrated in Figures 9 to 15 of the accompanying drawings.

45. A mass analyser constructed and arranged to operate substantially as hereinbefore described with reference to an as illustrated in Figures 9 to 15 of the accompanying drawings.

Fig.1.

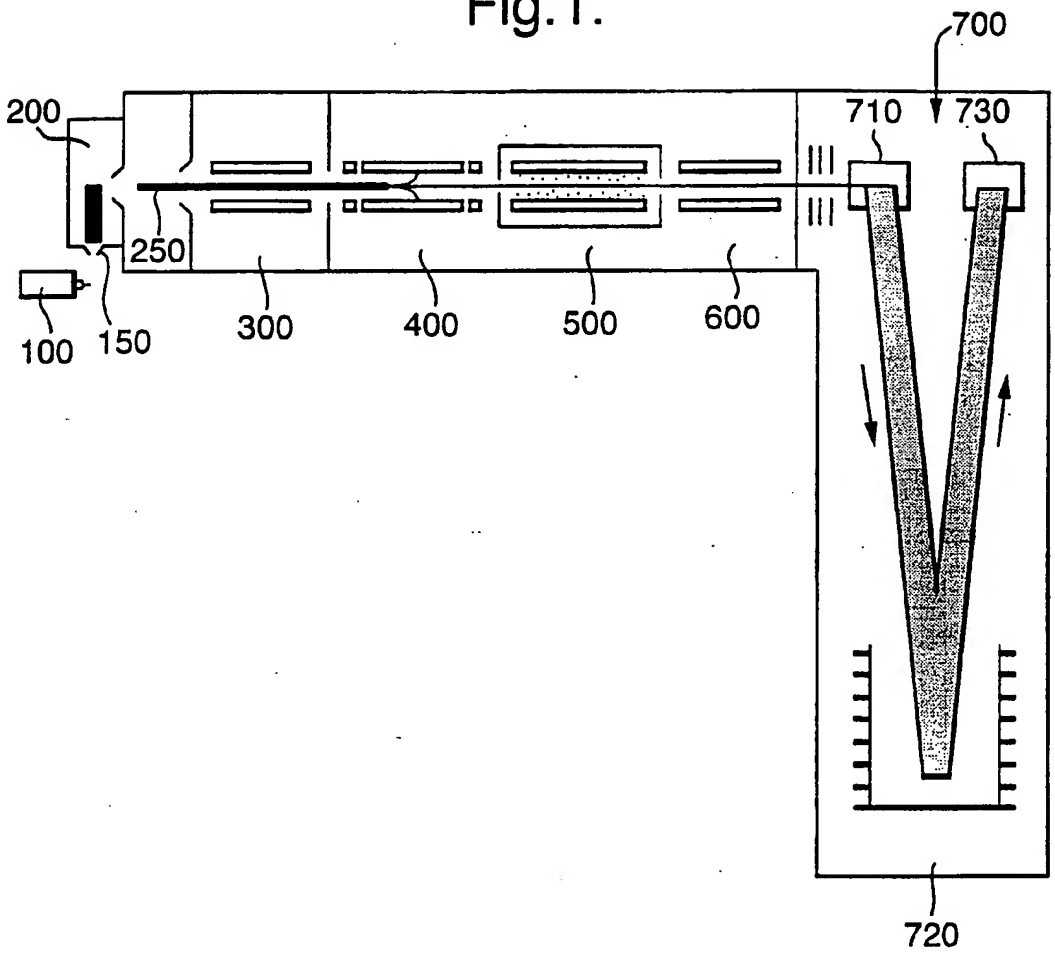


Fig.2. 100

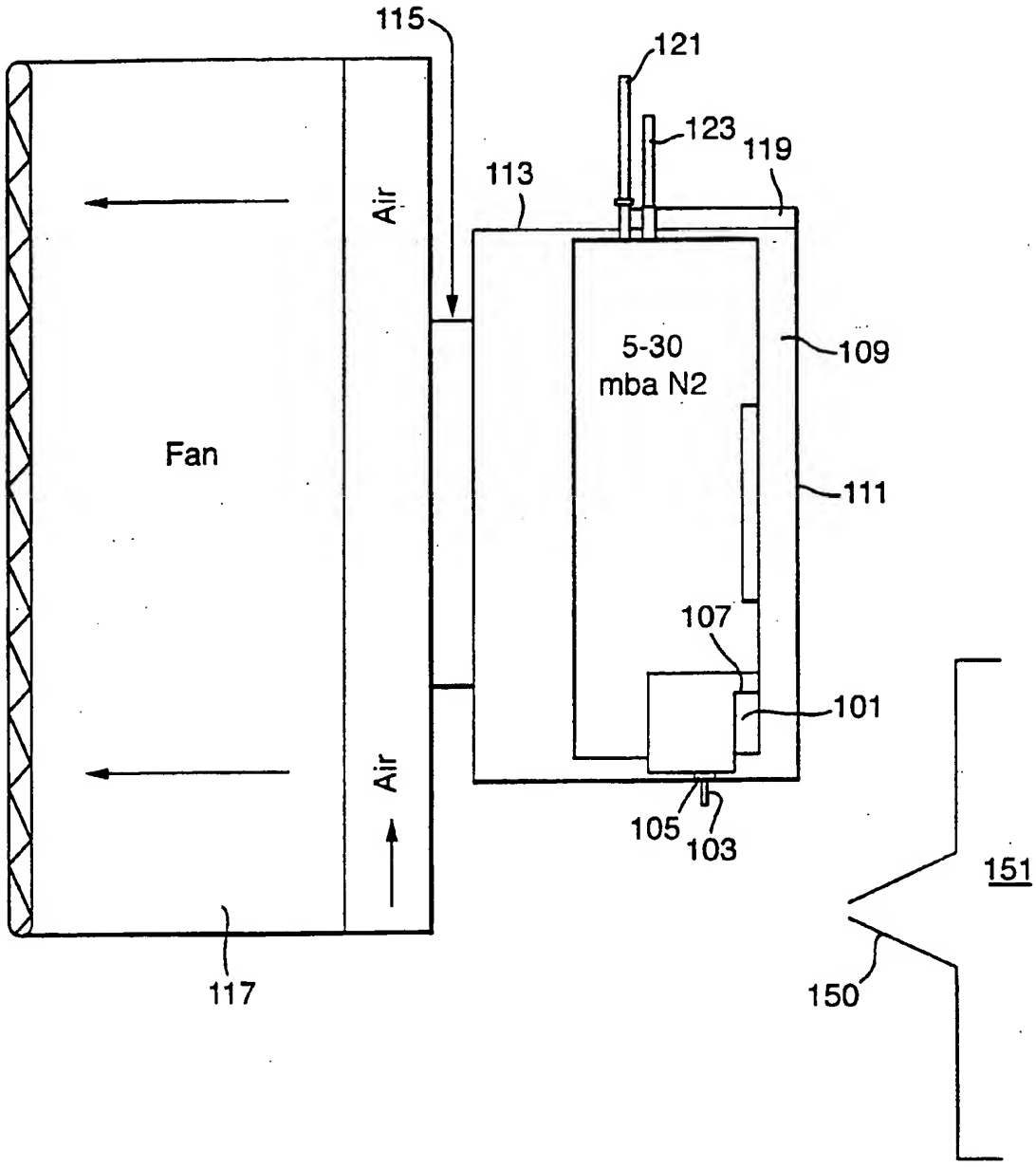
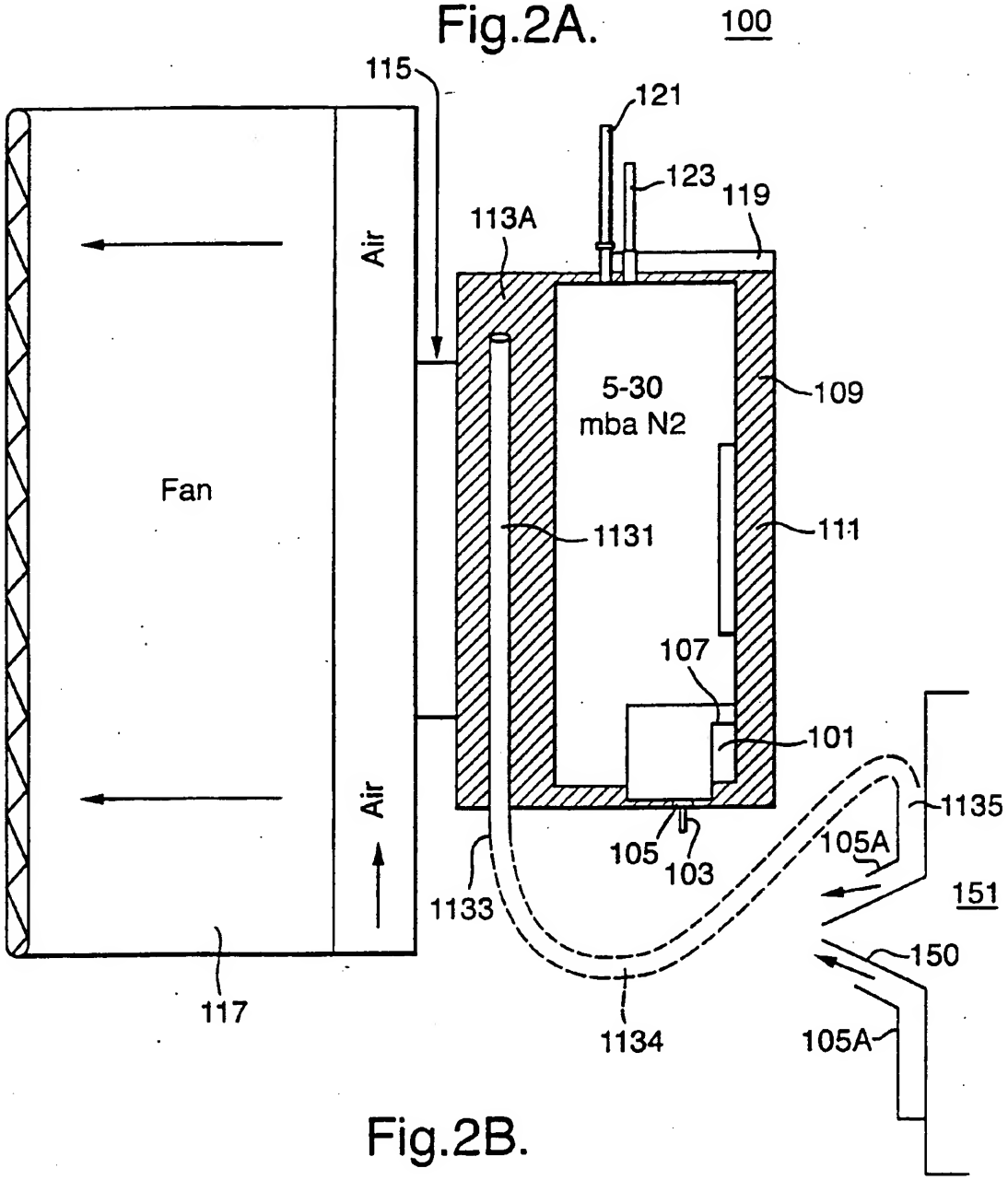

115
121
123
119
113
Air
5-30
mba N2
109
Fan
111
107
101
Air
105
103
151
150
117

Fig.2A.


100
115
121
123
119
113A
Air
Fan
5-30
mba N2
109
1131
111
107
101
Air
1135
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105
103
151
1133
150
117
1134
105A

Fig.2B.

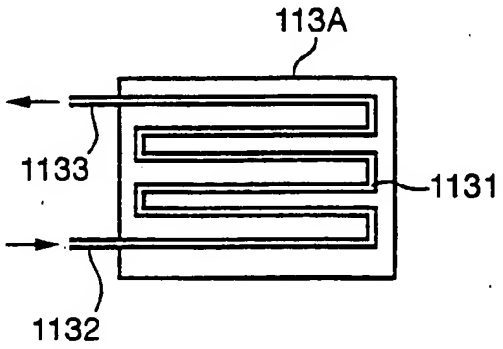

113A
1133
1131
1132

Fig.3.

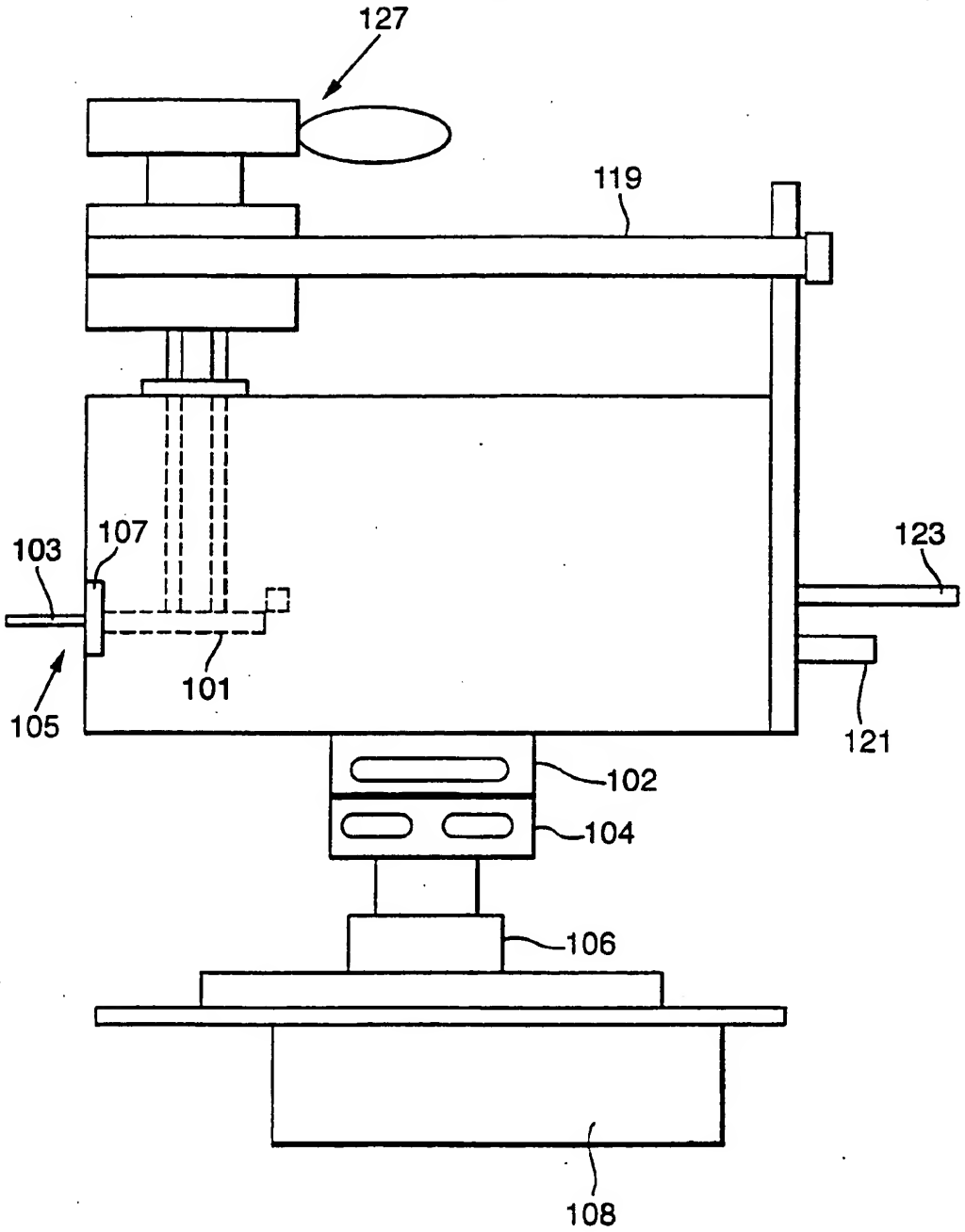


Fig.4.

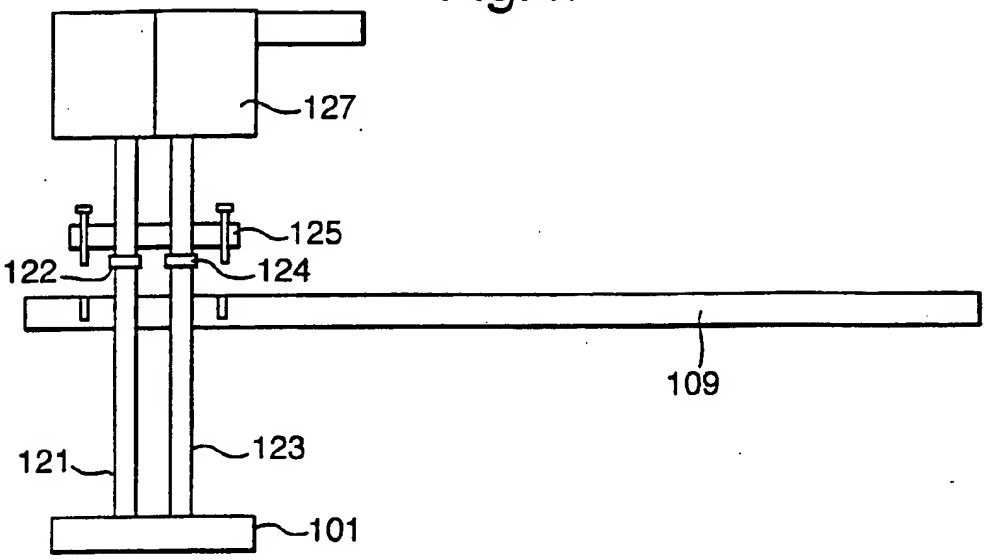


Fig.5.

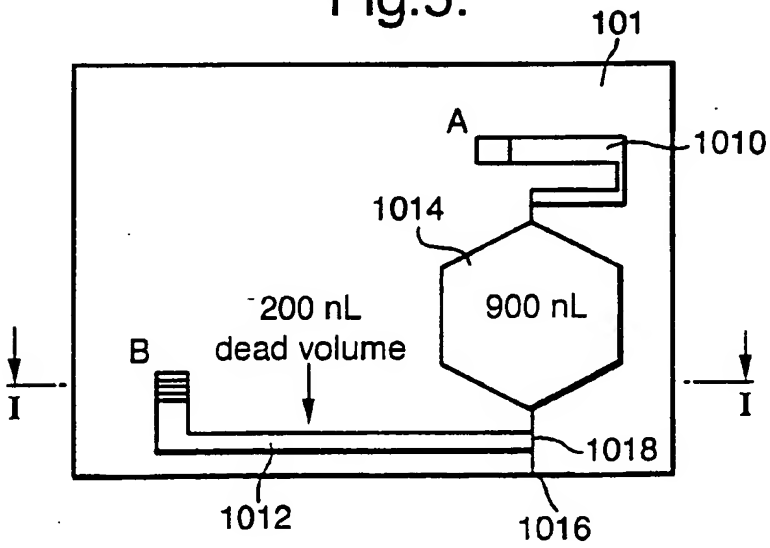


Fig.5A.

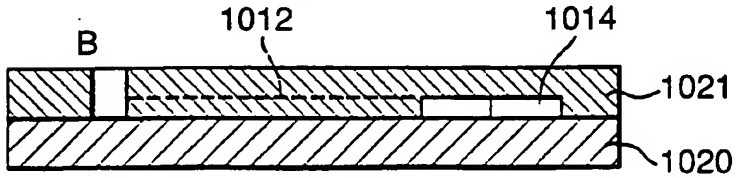


Fig.6.

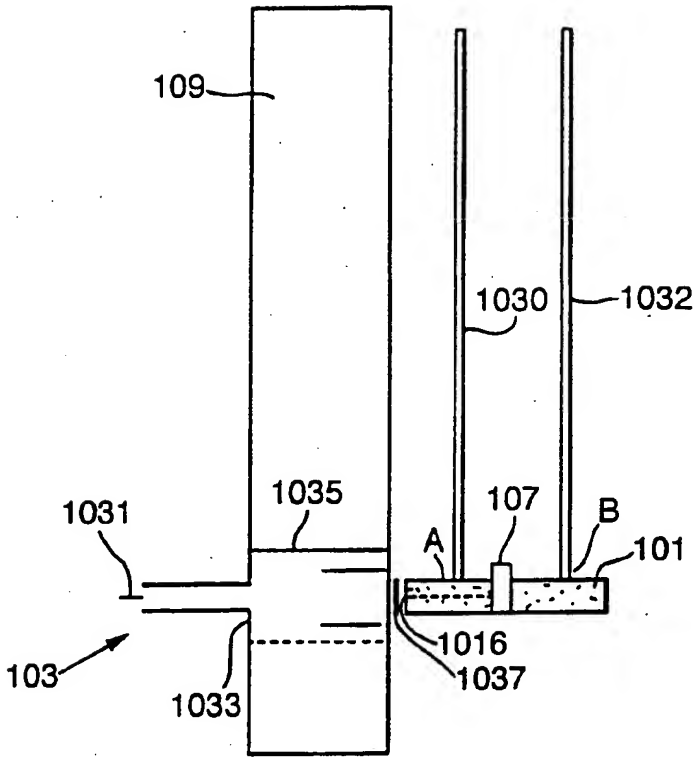
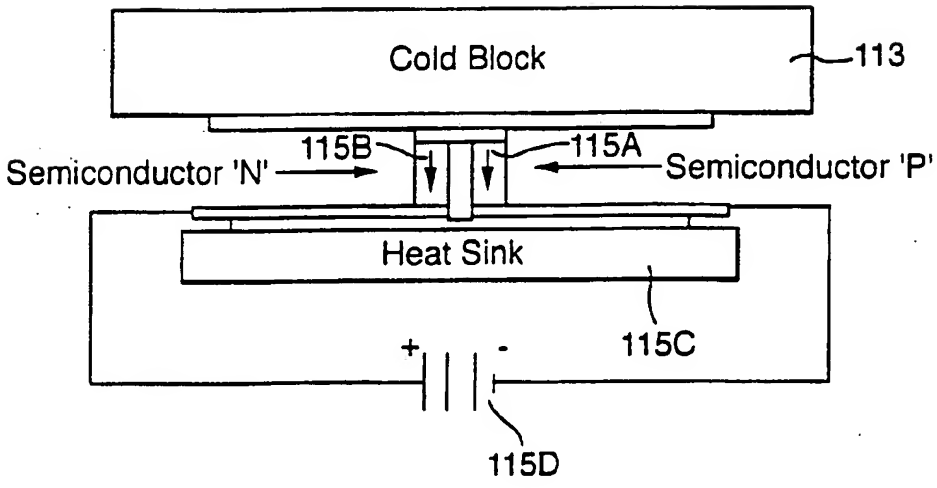

109
1030
1032
1031
1035
107
B
A
101
1016
1037
103
1033

Fig.7.


Cold Block
113
115B
115A
Semiconductor 'N'
Semiconductor 'P'
Heat Sink
115C
+
-
115D

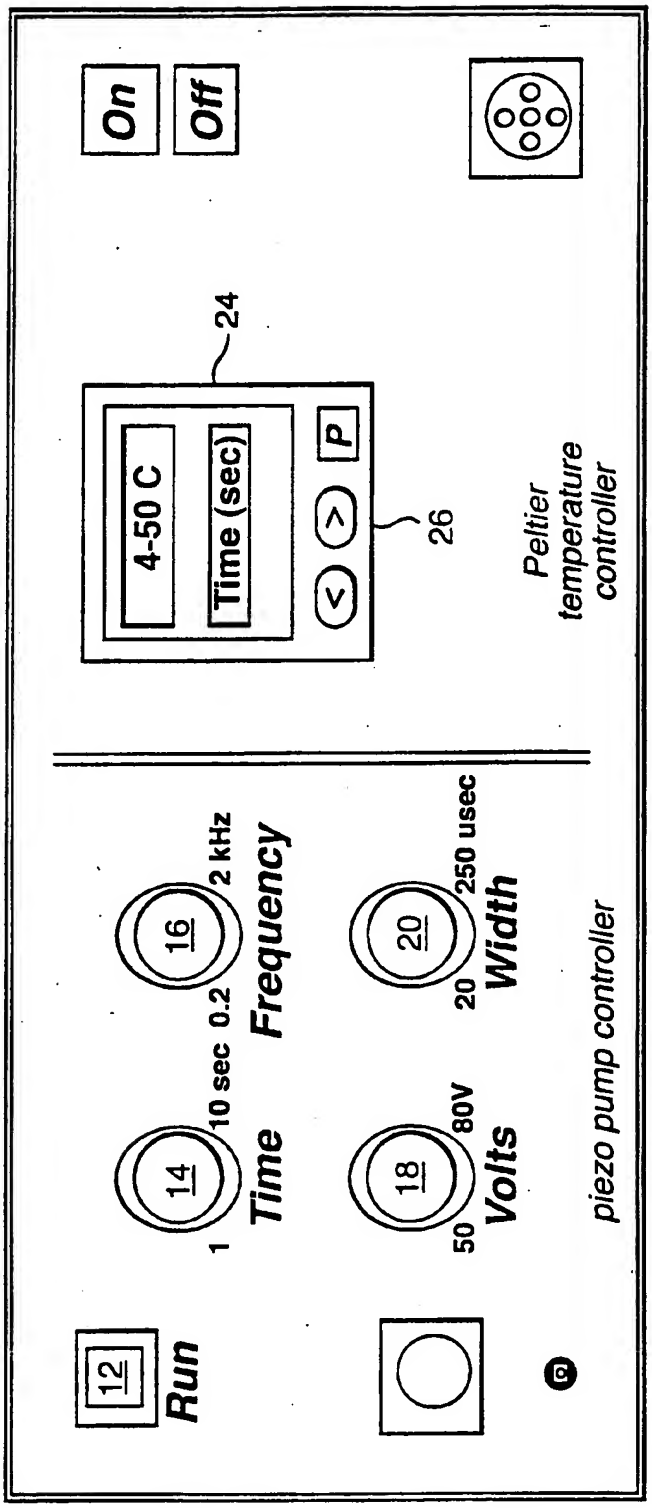

10
On
Off
24
4-50 C
Time (sec)
P
26
Peltier temperature controller
12
Run
14
10 sec
1
Time
16
2 kHz
0.2
Frequency
18
80V
50
Volts
20
250 usec
20
Width
piezo pump controller

Fig.8.

Fig.9.

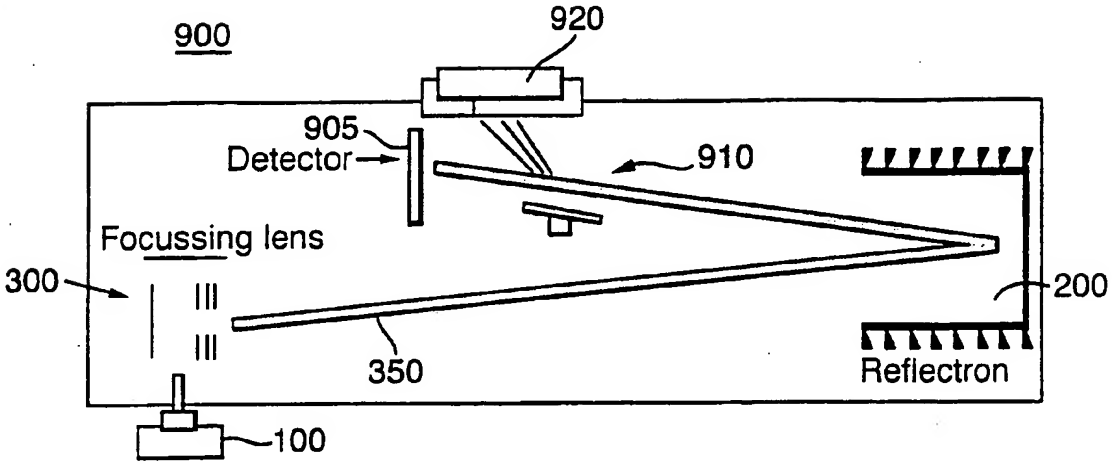

900
920
905
Detector
910
Focussing lens
300
200
350
Reflectron
100

Fig.10.

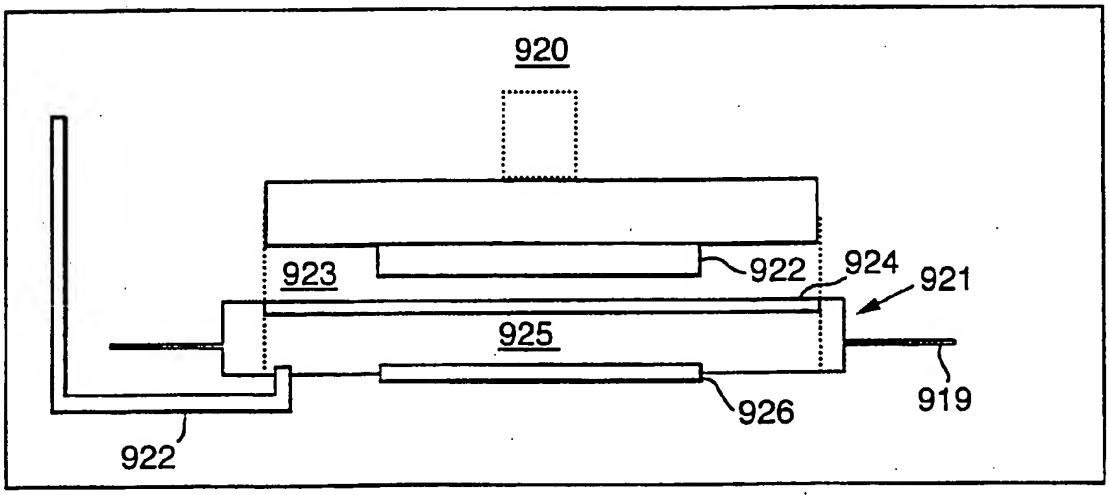

920
923
922
924
921
925
926
919
922

Fig.11.

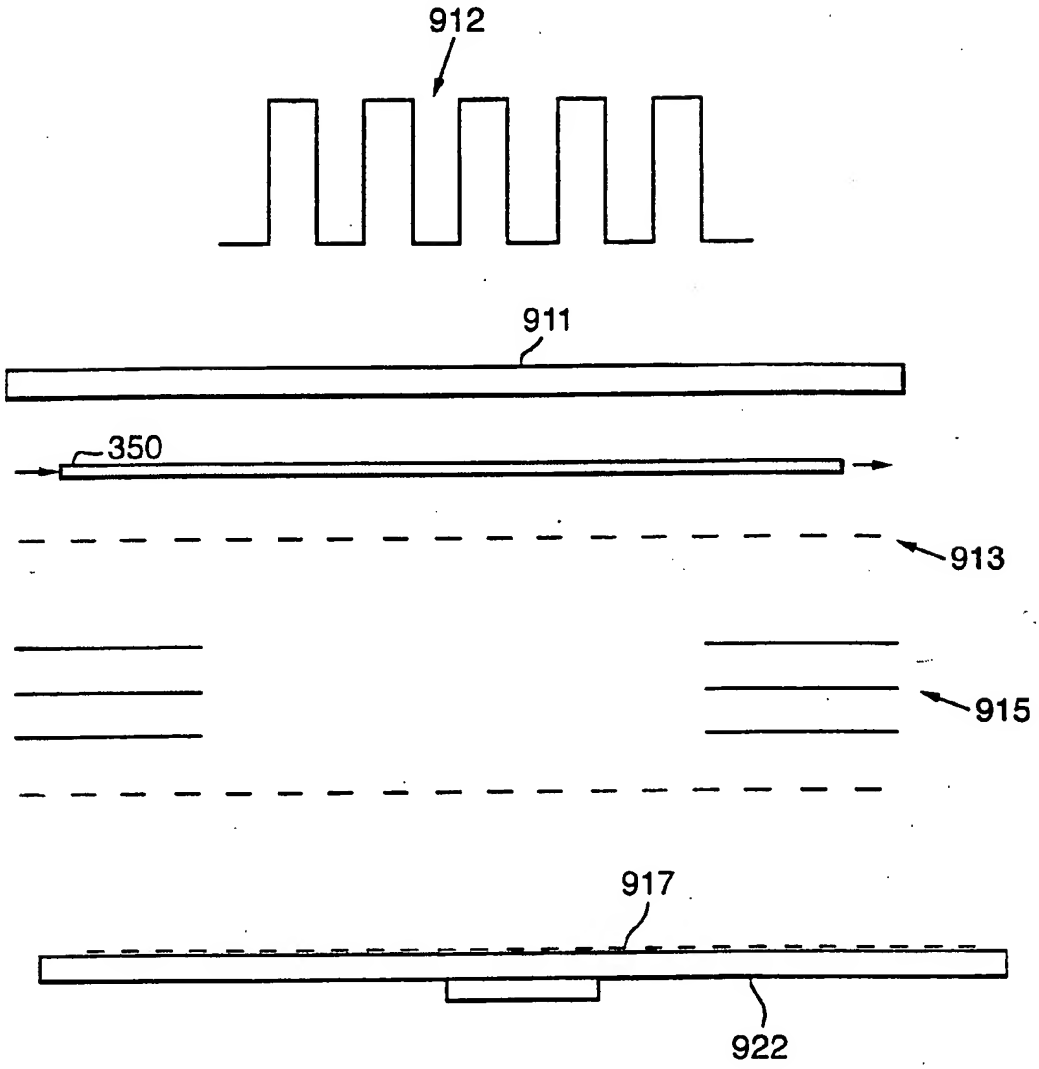
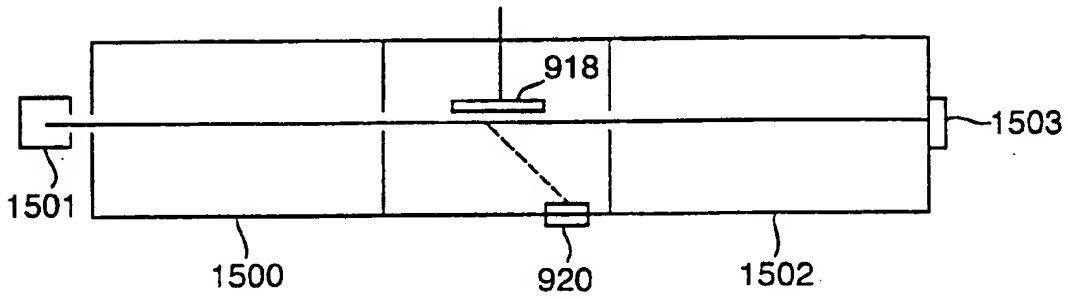


Fig.15.



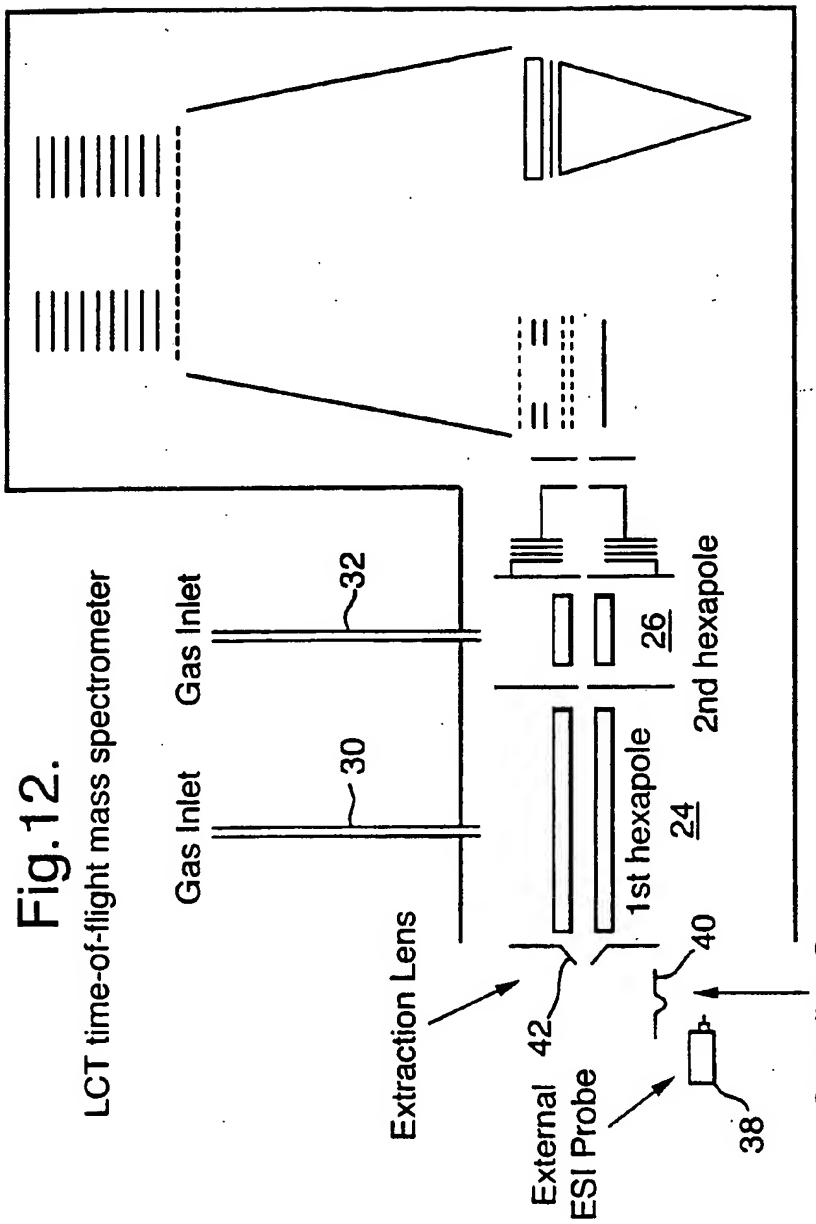
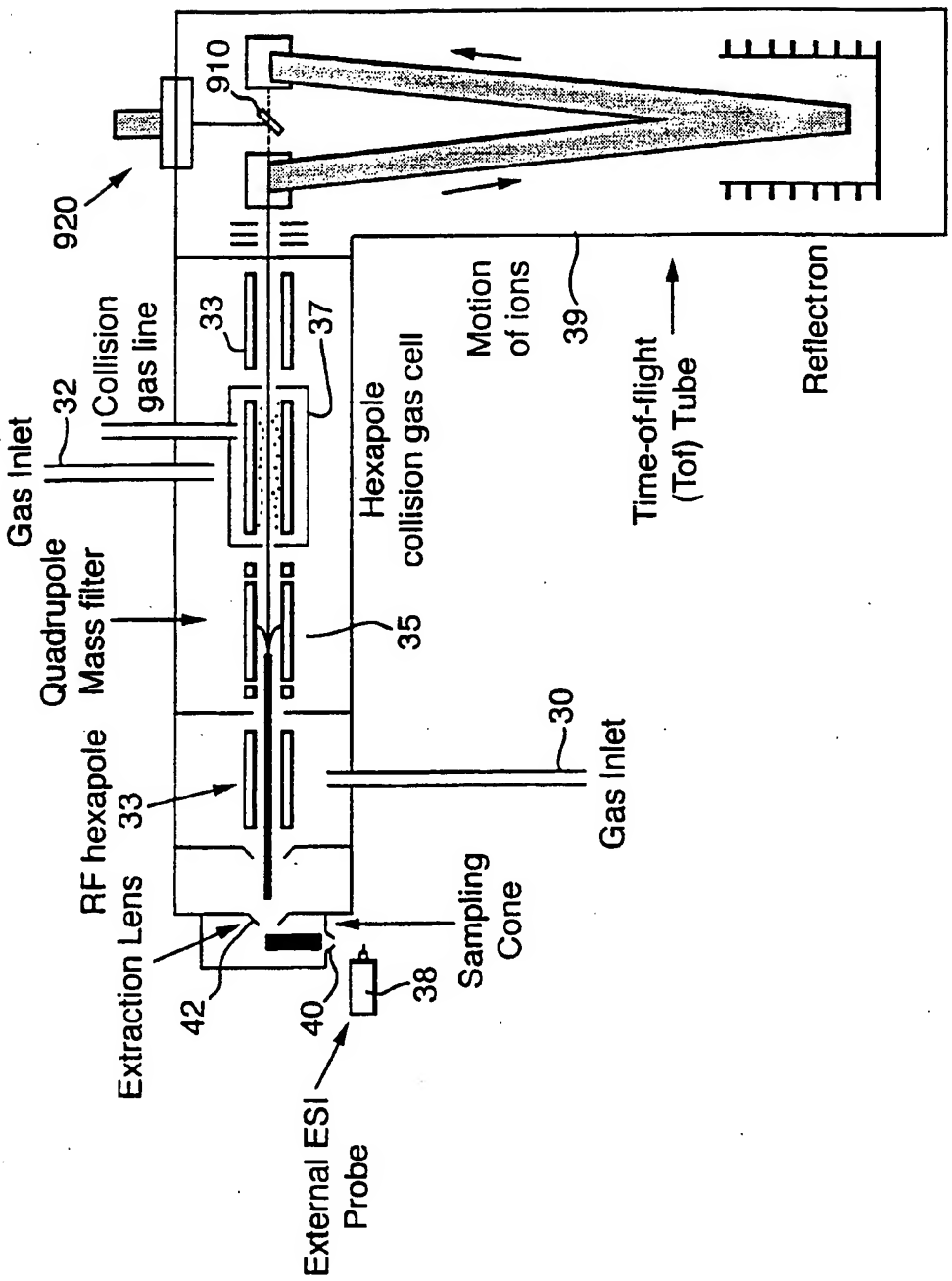


Fig.12.
LCT time-of-flight mass spectrometer

Fig.13.


910
920
Collision gas line
32
Gas Inlet
33
37
Hexapole collision gas cell
Motion of ions
39
Time-of-flight (Tof) Tube
Reflectron
Quadrupole Mass filter
35
RF hexapole
33
30
Gas Inlet
Extraction Lens
Sampling Cone
42
40
38
External ESI Probe

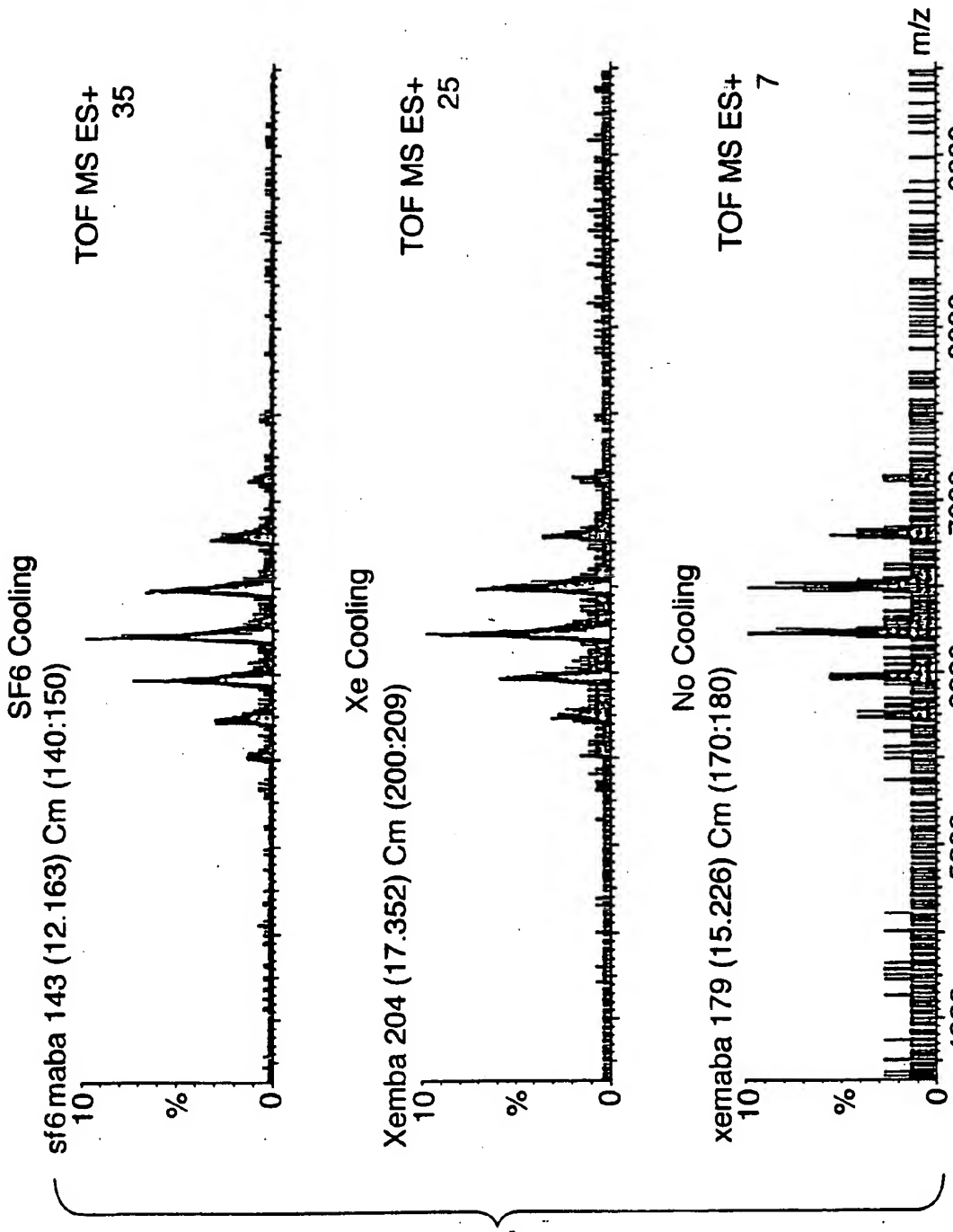

SF6 Cooling
sf6maba 143 (12.163) Cm (140:150)
TOF MS ES+
35
Xe Cooling
Xemba 204 (17.352) Cm (200:209)
TOF MS ES+
25
No Cooling
xemaba 179 (15.226) Cm (170:180)
TOF MS ES+
7
10
%
0
4000
5000
6000
7000
8000
9000
m/z

Fig.14.

Fig.16(a).

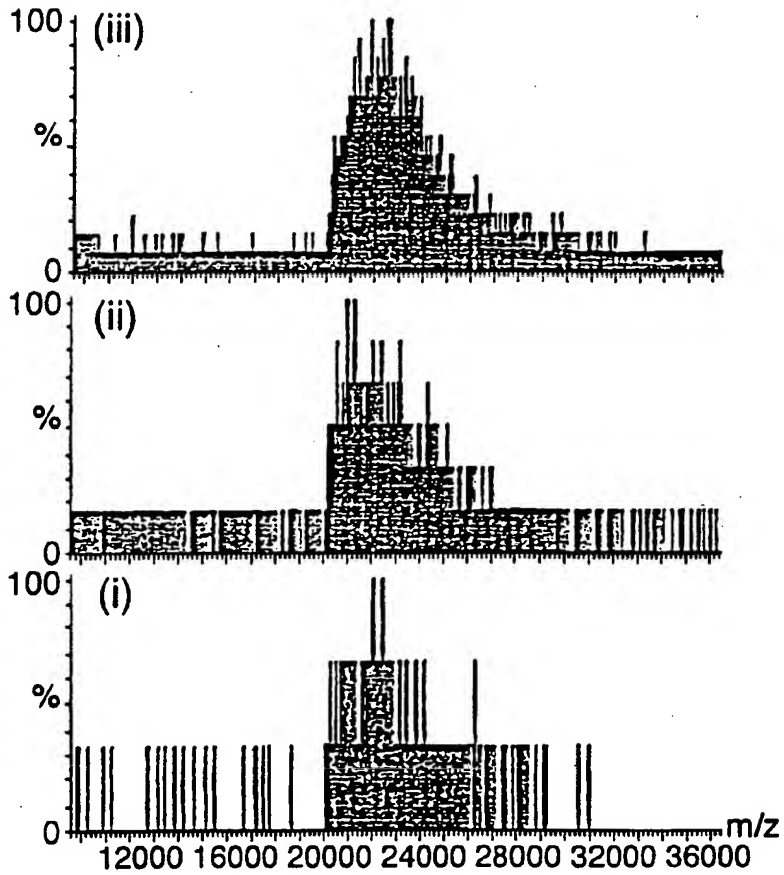
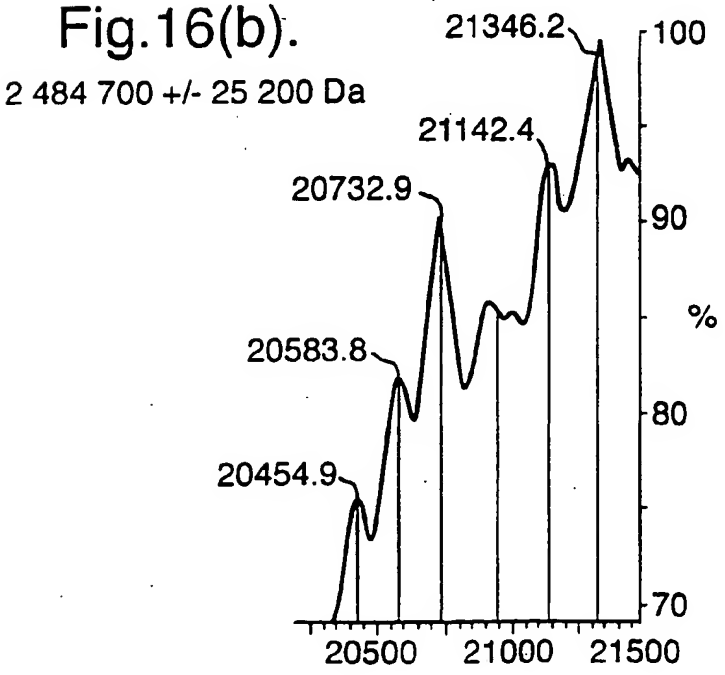


Fig.16(b).



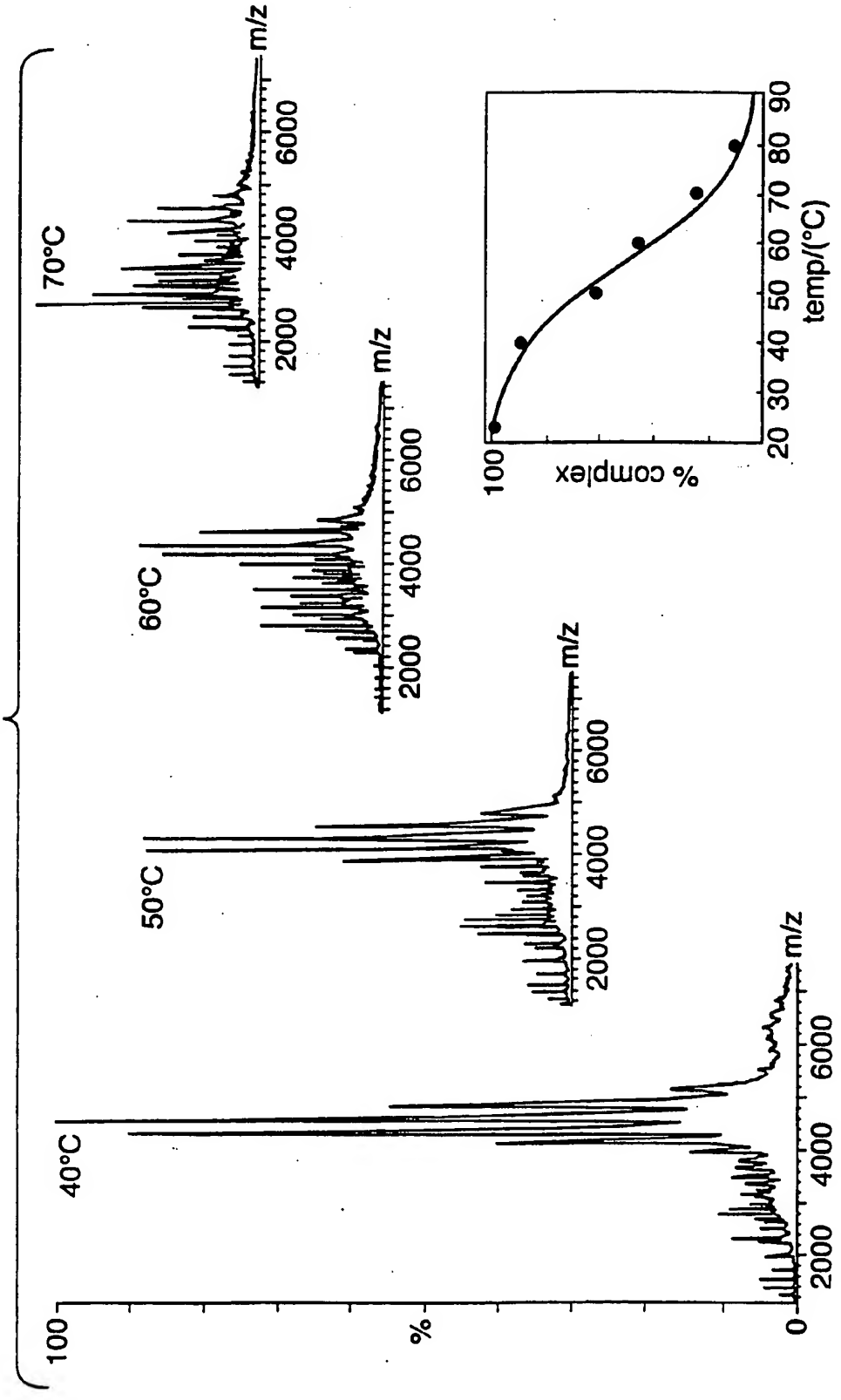

70°C
60°C
50°C
40°C
m/z
2000
4000
6000
100
%
0
% complex
temp/(°C)
20 30 40 50 60 70 80 90

Fig.17.